Interlab Study 2016 Notebook-Megan Satyadi

8/27/16

Transformation of Devices 1, 2, and 3, Positive Control, and Negative Control with DH5a

- Using C2989K (E. coli K, electrocompetent) from New England Biolabs

1. Buffer/water for the plasmids from the Interlab 2016 kit had evaporated, added 100uL of ddH20 and resuspended vigorously.
2. Thaw 25uL (for each transformant) of C2989K (New England Biolabs) for 10 minutes on ice.
3. Add 5uL of each plasmid to an aliquot of competent cells. Flick tube to mix.
4. Add cells/DNA mixture to .2cm cuvettes, keep on ice.
5. Shock cells at the following settings:
    a. 1.8kV
6. Add 975uL of warmed SOC to the cuvette, pipetting up and down twice before transferring to a culture tube.
7. Rescue cells for 1 hour at 37C and 200RPM.
8. Following rescue, spin cells down at 6000RPM for 4 minutes. Discard supernatent.
9. Resuspend in 101uL of fresh SOC. Make a 1:100 dilution.
10. Plate 100uL of each 1:1 and 1:100 on chloramphenicol-agar plates.
11. Incubate for 16 hours at 37C.

LUDOX OD_{600} Calibration

- Instrument: Tecan M1000 Plate Reader

1. Add 100 µl LUDOX into wells A1, B1, C1, D1
2. Add 100 µl of H_2O into wells A2, B2, C2, D2
3. Measure absorbance 600 nm of all samples in all standard measurement modes in instrument

	LUDOX	H2O
Replicate 1	0.0533	0.0493
Replicate 2	0.056	0.053
Replicate 3	0.0572	0.0493
Replicate 4	0.056	0.048
Average	0.055625	0.0499
Corrected Abs_{600}	0.005725	
Reference OD_{600}	0.01475	
Correction Factor	2.576419214	

FITC Solution Preparation

1. Spin down FITC stock tube to pellet.
2. Resuspend FITC in 1ml of 1x PBS.
3. Incubate at 42C for 4 hours
4. Dilute the 2x FITC stock solution in half with 1xPBS to form a 1x FITC solution (250uM)

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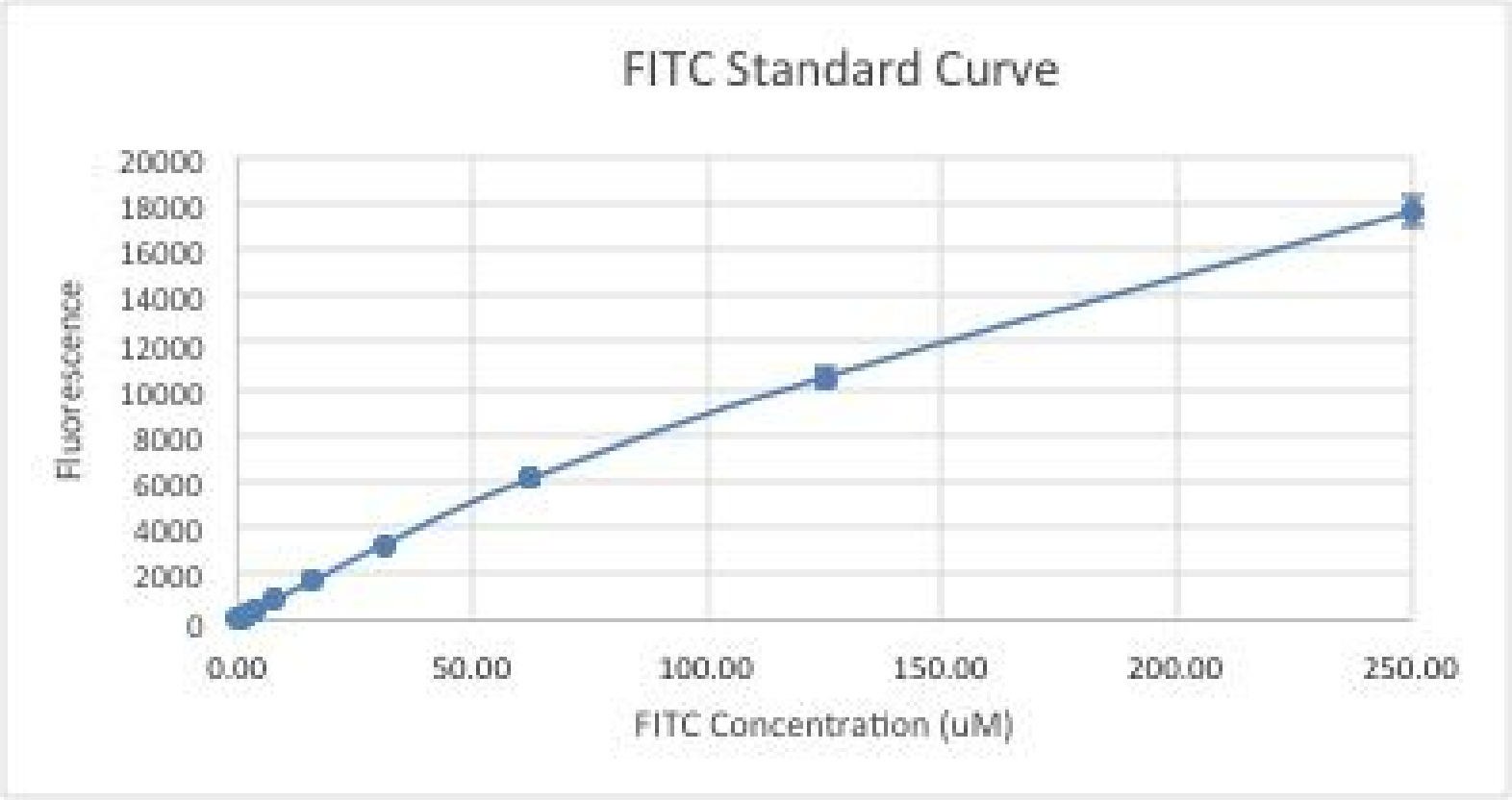
FITC Standard Curve

- Instrument: Tecan M1000 Plate Reader

1. Add 100 µl of PBS into wells A2, B2, C2, D2....A12, B12, C12, D12
2. Add 200 µl of FITC 1x stock solution into A1, B1, C1, D1
3. Transfer 100 µl of FITC stock solution from A1 into A2.
4. Mix A2 by pipetting up and down 3x and transfer 100 µl into A3...
5. Mix A3 by pipetting up and down 3x and transfer 100 µl into A4...
6. Mix A4 by pipetting up and down 3x and transfer 100 µl into A5...
7. Mix A5 by pipetting up and down 3x and transfer 100 µl into A6...
8. Mix A6 by pipetting up and down 3x and transfer 100 µl into A7...
9. Mix A7 by pipetting up and down 3x and transfer 100 µl into A8...
10. Mix A8 by pipetting up and down 3x and transfer 100 µl into A9...
11. Mix A9 by pipetting up and down 3x and transfer 100 µl into A10...
12. Mix A10 by pipetting up and down 3x and transfer 100 µl into A11...
13. Mix A11 by pipetting up and down 3x and transfer 100 µl into liquid waste.
14. Repeat in Rows B, C, and D.

(uM)	250.00	125	62.5	31.25	15.625	7.8125	3.90625	1.953125	0.9765625	0.48828125	0.244140625	0
replicate 1	17782	10929	6402	3351	1730	885	450	269	177	124	111	14
replicate 2	17583	10765	6178	3288	1720	869	457	263	172	122	105	14
replicate 3	16795	10065	5996	3298	1596	845	439	247	148	97	85	14
replicate 4	18322	10230	5929	3172	1582	811	413	237	144	99	89	14
Mean	17620	10497.	6126.2	3277.2	1657	852.5	439.75	254	160.25	110.5	97.5	14

	.5	25	5	5								
SD	632.729802	414.873776	211.8086794	75.41606814	78.83315715	32.18177538	19.31105038	14.65150732	16.66083231	14.47987109	12.47664485	0



Inoculation of Samples

- Picked 2 colonies off each plate and inoculated into 5mL of LB+5uL 1000X chloramphenicol

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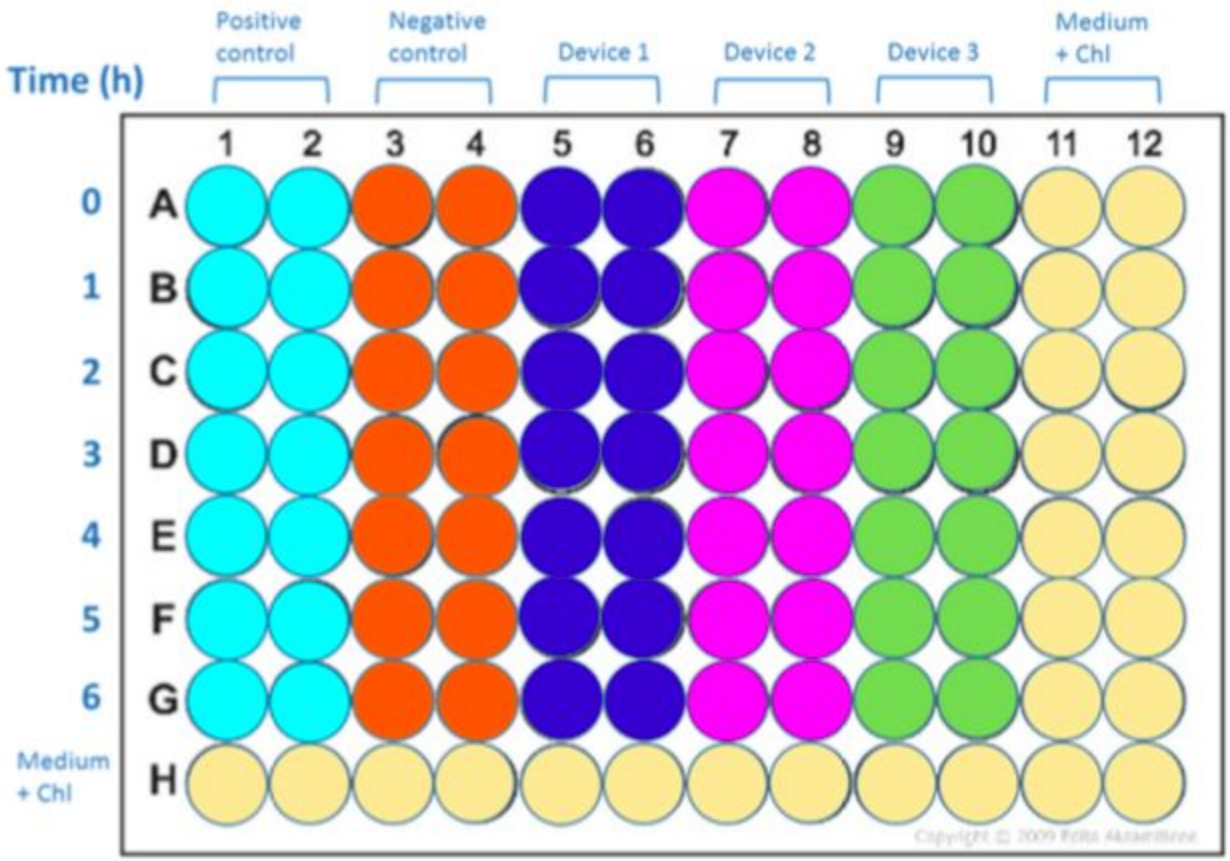
Cell sampling

1. Set your instrument to read OD600 (as OD calibration setting)
2. Measure OD600 of the overnight cultures
3. Record data in your notebook
4. Import data into Excel (normalisation tab) Sheet_1 provided
5. Dilute the cultures to a target OD_{600} of 0.02 (see the volume of preloading culture and media in Excel (normalisation tab) Sheet_1) in 10 ml 0.5x TB medium +Chloramphenicol in 50 mL falcon tube (if using cuvettes, you can use 100 ml in a 500 ml shake flask).
6. Incubate the cultures at 37°C and 220 rpm.
7. 100 µL (1% of total volume) samples of the cultures at 0, 1, 2, 3, 4, 5, and 6 hours of incubation (if using cuvettes, remove 1 ml from 100 ml culture).
8. Place samples on ice.
9. At the end of sampling point you need to measure your samples (OD and Fl measurement), see the below for details

Measurement and Assay

- use the same instrument settings that you used when measuring the FITC standard curve and same volume (100uL)
- Samples should be laid out according to Fig. 2. Pipette 100 µl of each sample into each well. Set the instrument settings as those that gave the best results in your calibration curves (no measurements off scale).



After pipetting all samples in the format shown in the figure above, measured fluorescence with the Tecan M1000 Plate Reader.